Prevalence of Bovine Tuberculosis (BTB) in Cattle Using Antibody ELISA in Seven Counties of Kenya

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SUMMARY

Bovine tuberculosis is an important zoonotic disease whose eradication has proved problematic due to the challenges in effective screening and diagnosis. A study to determine the presence and prevalence of bovine tuberculosis antibodies in seven counties of Kenya was carried out between August 2013 and December 2013. The study used bovine sera that were collected from seven counties in Kenya between 2011 and August 2013 and stored at the Central Veterinary Laboratories (CVL), Kabete Nairobi. The study utilized a newly developed antibody ELISA Kit. A total of 644 bovine serum samples were tested using MPB70 and MPB83 recombinant proteins as capture antigens. Data was recorded in Microsoft excel and exported to SPSS 16.0 version for analysis. The study showed a prevalence of 3.57% (23/644) with Wajir County having the highest prevalence of 4.7% (4/85). On Chi-square and fishers exact test there was no significant association between BTB infection with age (P= 0.05507), Breed (P=0.4111) and sex (P=0.2354). This study has documented the prevalence of BTB in cattle for the first time in Kenya utilizing a more specific antibody ELISA. This method of diagnosis presents a quicker and a cheaper way to the screening of BTB in live animals. This study also concludes that BTB is present in Kenyan cattle populations and recommends a survey of all counties and wildlife reserves to map out a comprehensive prevalence status of the whole country.
Key words: Cattle, MPB70, MPB83**,** *Mycobacterium bovis,* prevalence.

INTRODUCTION

Bovine tuberculosis (BTB) is a chronic infectious disease of cattle caused by *Mycobacterium bovis*. It is usually characterized by development of avascular granulomatous nodules known as tubercles. Many body tissues can be affected, however lesions are frequently seen in the lymph nodes of the head and thorax, lungs, liver, and peritoneum among other tissues (Clerke, 1998). *M. bovis* is virulent for cattle but can infect other animals and humans causing disease and pathology similar to *M. tuberculosis*, which is mainly pathogenic for man (Kaneene and Pfeiffer 2006; Thoen *et al.'* 2006). *M. caprae* has been identified as a cause of BTB in some parts of central Europe and its infection is not substantially different from that caused by *M. bovis* with similar tests being used for its diagnosis (Naranjo *et al.,* 2008).

The genus *Mycobacterium* consists of more than 100 species. Most of these are found in the environment and are not normally pathogenic to humans or animals however a small number of them are pathogenic to humans and various species

of animals (Sam *et al.,* 2011). BTB is endemic in many areas of the world and poses significant losses directly by reduced cattle productivity, control and eradication programs as well as being a zoonotic infection in man (Steele, 1995). In the subtropical African countries particularly, there is very little or no presence of control programmes posing a high risk of human infection and affecting international trade on livestock products (Biet *et al.,* 2005). The prevalence of *M. bovis* has been reported at 2.05 % for the first time among slaughter cattle in Kenya, in a study utilizing culture and PCR confirmation. (Gathogo *et al.,* 2012).

BTB has proved difficult to eradicate in various countries due to the presence of wildlife reservoirs, increased animal movement in pastoral regions and limitation of diagnostic and screening methods (Schiler *et al.,* 2010). Due to the potential zoonotic importance of BTB and little information available about its impact, it is necessary to build information especially in developing countries. Diagnosis of bovine tuberculosis remains extremely challenging and there is currently no one single test that can fulfil all the criteria necessary to identify all infected animals (Schiler *et al.,* 2010b). These limitations continually call for establishment of status information as the starting point. Application of ante mortem diagnostic tests like the IDEXX ELISA to determine the prevalence can be a feasible and a cheaper method to apply. Antigens such as ESAT-6, MPB70 and MPB83 have been evaluated for ELISA showing a good degree of reliability. The shared antigen of *M. bovis* cross reacts to *M. kansasii*, *M. szulgai* and *M. marinum* which are not pathogenic to bovine (Amadori *et al.,* 2002; Farias *et al.,* 2012). There is very little information about the prevalence status of bovine tuberculosis in Kenya and therefore an urgent need to bridge this gap. This therefore provided overview information about the status of BTB. This test has been shown to achieve sensitivities of more than 60% and a specificity of 98% which is better than earlier ELISA tests. The test has a potential of being used on milk and can also be complementary to other BTB tests (Waters *et., al* 2011).

MATERIALS AND METHODS

Study Area and design

Kenya has 47 counties all of which practice livestock farming at varying levels and systems. The study focused on seven counties due to availability of samples and accompanying proper records. These include, Likipia, Kilifi, Taita taveta, Wajir, Kajiado, West pokot and Kwale. It was a cross sectional study to estimate the prevalence of bovine tuberculosis utilizing a new antibody ELISA through the use of bovine serum. Samples were obtained from the Central Veterinary Laboratories (CVL) Serum bank. The samples were collected from the different counties between the years 2011 and August 2013 for surveillance by the department of veterinary services.

Sample size determination

The minimum sample size for cross-sectional survey was calculated using the formula by Dohoo *et al* (2003) and were 87 as shown below:

$$n = \frac{(1.96)^2 p(1-p)}{d^2}$$

Where d is the required precision, was assumed at 95% (error margin of 5%), p is the anticipated prevalence assumed to be 6%. The n is the sample size calculated at 87, however it was increased to 644 due to availability of test kits.

Sample analysis using the Antibody ELISA

The test is an indirect ELISA that detects presence of antibody to *M. bovis* in bovine serum. The test kits manufactured by IDEXX Technical Services (USA). This test has a two hour procedure protocol and utilizes recombinant MPB70 and MPB83 proteins as capture antigen in the 96 wells micro-titer plates.

Test procedure

Antigen coated plates sufficient for planned samples were tested at a time and withdrawn from the desiccated bag. 100 micro liters of the diluted negative control, positive control and serum samples were dispensed in the wells. The wells were covered and incubated in 18-26°C for 60 minutes then washed 3 to 5 times with 300 micro liters of wash solution avoiding drying in between washes. A total of 100 µl of the conjugate was then dispensed into the wells, covered, incubated for 30 minutes then washed 3 to 5 times as described previously. The substrate was then dispensed into the wells at 100 micro liters, covered and incubated for 15 minutes. Up to 50 µl of the stop solution was then dispensed last into the wells and absorbance read immediately at 450nm on an ELISA micro- titer plate reader.

Interpretation of the ELISA results

The optical density for the positive control mean must be greater than or equal to 0.3 while the negative control mean must be less or equal to 0.2. The presence or absence of antibody to *M. bovis* is determined by calculating the sample to positive (S/P) ratio for each sample. S/P greater or equal to 0.30 are considered positive for *M. bovis* antibodies while samples with less than 0.30 are considered negative for *M. bovis* antibodies. Data from ELISA results was entered into Excel 2007 (Microsoft Corporation, USA). The data was then exported to SPSS 16.0 version for analysis.

RESULTS

Samples descriptive and prevalence of Bovine tuberculosis per county

A total of 644 serum samples were analysed with 23 out of 644 being positive to ELISA test. Of the samples tested, 112 (17.4%) were males and 532(82.6%) females. 1.8% (2/112) of serum samples from male animals tested positive for antibodies against *M. bovis* while 3.9% (21/532) of the serum samples from females tested positive. The BTB seroprevalence ranged from 0% in Kilifi county to 4.7% in Wajir (Table 1). Number of samples across the counties ranged from 45 (7.0%) to 276 (42.9%). The table below shows the distribution of samples in all the seven Counties.

Table 1. Distribution of BTB positive sample per county

County	Total samples	Number positive	Prevalence (%)
Laikipia	276	11	4.0
Wajir	85	4	4.7
Taita Taveta	60	2	3.3
Pokot	68	3	4.4
Kwale	45	1	2.2
Kilifi	64	0	0.0
Kajiado	46	2	4.4
Total	644	23	3.6

Prevalence of bovine tuberculosis per unit

There were a total of fifty nine sampling units across the seven counties from which the samples were obtained. These units were ranches for Laikipia while in other

counties they were villages from different locations across each county**.** In Kajiado the units were not recorded and were regarded as one, Laikipia had the highest farm prevalence of 58.6% (7/12) followed by Pokot County which had 40% (2/5) as shown in Table 1.

About 1.8% (2/112) of serum samples from male animals tested positive for antibodies against *M. bovis* while 3.9 % (21/532) of the serum samples from females tested positive. On Fisher exact test, there was no significant association between sex of the animal and ELISA outcome (p= 0.4111). Likewise on Chi-square test, there was no significant association between Age of the animal and ELISA outcome (p= 0.05507).

Table 2. Prevalence of *M. bovis* by units of sampling

County	Total Units	Positive Units	Negative Units	Prevalence (%)
Laikipia	12	7	5	58.33
Pokot	5	2	3	40.0
Wajir	11	4	7	36.4
Kajiado	1	2	0	100.0
Kwale	9	1	8	11.1
Kilifi	12	0	12	0.0
Taita Taveta	9	2	7	22.2
All counties (Total)	59	18	42	30.5

DISCUSSION

The use of antibody ELISA in the diagnosis of BTB has not been widely applied in many parts of the world. TSTs have been the most common methods of ante mortem screening cattle for a long period and in many areas of the world. This study was performed using stored bovine serum samples to estimate and establish the prevalence of BTB in Kenya using antibody ELISA. The serum samples were collected for routine surveillance by the epidemiology section in the department of veterinary services. It's the first time this method is employed in Kenya for diagnosis of BTB. IDEXX *M. bovis* antibody ELISA's diagnostic performance has been evaluated in various studies and reports published in the past three years (Eyob *et al.,* 2014; Waters *et al.,* 2011). Capture antigens especially recombinant MPB70 and MPB83 have been used in antibody ELISA as alternative and or complementary diagnostic tools to improve detection of cattle that do not react to tuberculin skin test (Smith *et al.,* 2006). MPB83 and MPB70 are major antigens highly expressed by *M. bovis* and considerably less abundantly expressed by *M. tuberculosis* (Juarez *et al.,* 2001). MPB83 have been detected in cattle infected with *M. kansasii*, making it the only known *Mycobacterium* species cross reacting with the antigen (Green *et al.,* 2009; Waters *et al.,* 2006).

The overall prevalence recorded in this study was 3.6% in the seven counties with a range of 4.7% in the highest prevalent county to 0% the lowest county. Like many other countries in the region Kenya lacks enough data and reference record of BTB status. The area of focus was varied, ranging from areas of pastoral system of cattle keeping to subsistent small scale

farming regions with some counties bordering wildlife reserves. Published work in Kenya indicates 2.0% *M. bovis* prevalence among slaughter cattle in two abattoirs around Nairobi (Gathogo *et al.*, 2012). The origin of cattle in that study was 18 counties of Kenya covering 77% landmass. Another study indicates a prevalence of 10% in dairy and non-dairy herds around Nairobi area (Kang'ethe *et al.,* 2007). A study in Kenya investigating the epidemiology of bovine tuberculosis in the wildlife-livestock interphase in Masai Mara and Amboseli ecosystems showed a prevalence of 14.8% in wildlife compared to an overall prevalence of 2.3% in livestock (Lekolool, 2011). In Kenya, *M. Bovis* was reported in baboons feeding on abattoir offal (Sapolsky and Else, 1987).

Less than1% prevalence was reported in a study conducted around Dar es Salaam area using Single intradermal Tuberculin Test (SITT) with 6.8% being doubtful (Weinhaupl *et al.,* 2000). A Prevalence of 2% and a herd prevalence of 51% have been reported in cattle pastoral herds of Uganda's Karamoja region (Oloya *et al.,* 2006; Oloya e*t al.,* 2007). The different Counties showed different prevalence irrespective of the different number sampled per county.

The prevalence per county varied from 4.7 % (4/85), Wajir with the highest rate followed by Pokot County with 4.41% (3/68) to 0% in Kilifi County. This has also been observed and reported in other regions. In South American for example reports show the highest levels of Bovine tuberculosis occurrence in the surrounding areas of larger cities where intensive dairy production is most common (Szyfres, 1972). Large variations in Bovine tuberculosis occurrence within different regions of the same country have also been reported in Africa (Cosivi *et al.,* 1995). A similar occurrence is reported in other areas where herd prevalence varies from region to region. A study in Northern Ethiopia between 2007 and 2008 utilizing of CIDT indicated a herd prevalence of bovine tuberculosis was lower in outdoor than indoor production systems (Mohammed *et al.,* 2012).

Out of the total 644 samples tested, 1.8% of serum samples from male animals tested positive for antibodies against *M. bovis* while 3.9 % of the serum samples from females tested positive. On chi-square test, there was no significant association between the sex of the animal and the ELISA outcome. This was also similar for Laikipia County where sex as a factor was also analysed. A cross-sectional study conducted in Tanzania from 1994 to 1997, revealed that male cattle were significantly more affected by BTB than female animals (Kazwala *et al.,* 2001). Another across-sectional study from 2006 to 2007, in Uganda revealed significantly more females positive to the skin test than males (Inangolet *et al.,* 2008). There was no significant association between age of the animal and ELISA outcome. This means that the chances of an animal testing positive for BTB antibodies do not change with age. In epidemiological studies carried out in Zambia and Tanzania it was observed that the duration of exposure increases with age (Cook *et al.,* 1996; Cleaveland *et al.,* 2007).

Irish workers observed that calves were less likely to be positive reactors in both ELISA and tuberculin skin test than older animals (Griffin *et al.,* 1996). Animals have been reported to get infected at a young age, but only express the disease clinically when they are adults (Pollock and Neil, 2002). Despite this, researchers have not been able to demonstrate that a

true dormant state exists in cattle (Van Rhijn *et al.*, 2008).

The IDEXX ELISA test has shown a sensitivity range of between 63% to 98 % and specificity of 98% at the cut-off values established by the manufacturer (Waters *et al.*, 2011). A study done in Ethiopia indicated a marked difference of sensitivities between the manufactures given Cut-off (0.03) and a lower cut-off of (0.136) at 50% and 80% respectively without affecting the specificity significantly. The lower cut-off was determined using receiver operating characteristics (ROC) analysis using culture as a gold standard (Eyob *et al.*, 2014).

Conclusion Recommendations

This study showed that BTB is present in Kenyan cattle populations in six out of the seven counties studied. There is an urgent need to Survey all the counties for BTB status in order to advise on control policy. Age, sex and breed were not significantly associated with BTB infection in the seven counties. The study showed that the use of antibody ELISA is feasible, reliable, cheaper and less time consuming. Therefore a need employ it to conduct a study to determine a suitable IDEXX ELISA cut-off S/P Ratio for Kenyan condition alongside a gold standard.

There is a deficit on the available data on the prevalence of BTB in domestic animals. With the high numbers of human cases recorded in Kenya for the years 2007 and 2008, there is need to investigate if these infection are related to livestock sources and the risks involved. The performance of the ELISA test showed a strong agreement when compared to the tuberculin test which is used as the gold standard for screening TB on live animals.

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